Reference: *Biol. Bull.*, **145**: 492–508. (December, 1973)

RESPIRATORY VARIATION AND ACCLIMATION IN THE FRESHWATER LIMPET, *LAEVAPEX FUSCUS* [1]

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The North American "pond" limpet, *Laevapex fuscus* (C. B. Adams), is a member of the family Ancylidae; and the order Basommatophora (Basch, 1959, 1963 and Hubendick, 1964). It is one of the most highly evolved of the freshwater pulmonates in that the mantle-cavity is reduced and the main respiratory structure is a secondary gill formed from a double evagination of the left mantle-wall in the region of the rectum. General cutaneous exchange along with this "pseudobranch" allows *Laevapex* to be entirely aquatic in its respiration. It does not have to return to the surface periodically to renew air as in the majority of freshwater pulmonates. *Laevapex* is distributed throughout North America east of the Rocky Mountains in swamps, ponds, rivers, and lakes. It lives on rocks and other hard surfaces (Basch, 1959, 1963 and Hubendick, 1964).

The present work is concerned with the respiratory behavior of *Laevapex* and the correlation of this with both natural history and environmental variation. Earlier investigations involve the annual repiratory variation of three ancylid species: the European species, *Ancylus fluviatilis* and *Acroloxus lacustris* (Berg, 1951, 1952, 1953, and Berg, Lumbye and Okelman, 1958); and the North American stream limpet, *Ferrissia rivularis* (Burky, 1969, 1970, 1971). Like *Laevapex,* these ancylids all show reverse respiratory acclimation in winter-conditioned populations. The phenomenon of reverse acclimation appears to be paradoxically non-adaptive (Prosser, 1955). A major result of this study is a re-interpretation of the function of reverse acclimation in *Laevapex* within an appropriate ecological and environmental framework.

Specimens of *Laevapex fuscus* were collected bimonthly from three freshwater environments in upstate New York through the years 1970–1971. Concurrent studies of life-cycles, reproduction rates and bioenergetics (McMahon, 1972; see also McMahon, Hunter and Russell-Hunter, 1974) were made along with the assessments of respiration described in this report. Oxygen consumption rates were determined at both ambient and two standard temperatures for 100% oxygen tension on subsamples from each collection. For every 9 out of 10 of the seasonal samples, the effects of decreasing oxygen tensions at 20° C on rates of oxygen consumption were determined. In addition experiments were carried out on respiration rates before and after exposure to oxygen depleted water.

[1] This investigation was supported initially by National Institutes of Health grant number GM 11693, and then by National Science Foundation grant number GB 36757, both to Dr. W. D. Russell-Hunter.

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Methods

Three populations of *Laevapex fuscus* were investigated in this study. They occurred in Canandaigua Outlet (CAN) Canandaigua, New York (USGS map quadrangle, Canandaigua Lake, New York: 42°52′28″N, 77°16′08″W); Sterling Pond (FH) in Fair Haven, New York (USGS map quadrangle Fair Haven, New York: 43°20′30″N, 76°42′00″W); and the Erie Canal (ECF) just outside Fayetteville, New York (USGS map quadrangle, Syracuse East, New York: 43°02′50″N, 76°00′26″W). Large regular collections of *Laevapex fuscus* were taken at the CAN and FH sites from mid-August 1969 until late-December 1971. Erie Canal at Fayetteville was sampled regularly from June 1970 until late-November 1971.

Fair Haven and Erie Canal collections consisted entirely of specimens taken from rocks. Canandaigua Outlet samples came from rocks, bottles, metal cans and pieces of wood littering the bottom. At all three sites water temperatures varied between summer highs of 25–28° C and winter lows of 0–4° C (Fig. 1). During the warmer periods specimens of *Laevapex* were found on rock surfaces just within the oxidized surface layer of mud and organic debris. In the winter when water temperature is low, *Laevapex* migrates well down the rocks into very poorly oxygenated reducing mud and remains inactive until temperatures rise in the spring. Once ice forms in the winter oxygen tensions drop at all three sites due to diminished diffusion of oxygen from the surface (McMahon, 1972).

All respiration rates were monitored with Clark-type polarographic oxygen electrodes (Clark, 1956). The oxygen electrodes, respiration chambers and constant temperature apparatus was purchased from the Yellow Springs Instrument Company (Model-53). Respiration chambers were modified for snails according to the method of Burky (1969, 1971). Oxygen tension in the chambers was continuously recorded on a Honeywell Electronik-16 Strip Chart Recorder.

Specimens of *Laevapex fuscus* were returned to the laboratory in vacuum flasks and maintained at field temperature in an incubator. Respiration experiments were normally started within 24 hours of the collection. Rates were determined for one to four age-groups (premeasured as shell size-classes) intended as representative of the frequency distribution of the population sampled. The standard measurement for all samples was aperture length (AL), the greatest dimension anterior-posterior across the opening of the limpet shell. The class interval for each age group was ± 2.5 mm about a chosen aperature length within the range, one to eight millimeters (Snails were measured according to the method of Russell Hunter, 1953, 1961). Replicate experiments of the same size-classes were run occasionally as an indication of respiratory variance. After respiration measurements, experimental groups were taken to a constant dry weight in a 90° C oven (usully more than 12 hours). To assess shell biomass thirty or more snails were randomly selected from a summer sample at each site plus a winter sample at FH, and placed in 90° C pond water for one minute to separate each shell from its mantle tissue. For these separated shells, length and shell constant dry weight (90° C for three hours) were determined. Regressions were then computed for shell dry weights against aperture length (the longest dimension across the opening of the shell) for the ECF and CAN populations and both generations at FH.

Water used in the chambers was taken from the field at the time of collection, held at field temperature, and filtered twice before being used in any experiment.

Chamber water volume was always four milliliters. The oxygen probe was equilibrated at least twenty-five minutes in a blank chamber (*i.e.*, without limpets) before any experimental determinations were made.

The limpets were added to the experimental chamber once the water had stabilized at the experimental temperature and they remained nearly stationary after attachment for the course of the experiment. All sets of respiratory experiments consisted of runs at three temperatures (at field ambient temperature and at 10° C and 20° C). Although the first respiration run in each experimental sequence was done at field ambient temperature the temperature for the second run was chosen with reference to the recent environmental experience of the population.

Each experiment began at a saturation level of oxygen concentration assumed to be equal to that for distilled water at the experimental temperature and pressure. In the majority of runs, all rates were determined from the records of a continuously monitored change in oxygen concentration, either from 100% to 90%, if that 10% reduction was accomplished in less than 30 minutes, or from the reduction from 100% that actually occurs in 30 minutes.

Other experiments involved continuous monitoring at 20° C from 100% oxygen down to a tension at which respiration apparently stopped. For these more extensive runs, respiratory rates were normally calculated for every 10% change in concentration down to 30%, but, where rapid changes in rate occurred, the rates were determined at every five per cent decrease in concentration. In addition, another four series respiratory experiments were conducted. First, temperature acclimation was assessed in the laboratory by holding animals in an incubator at 20° C, after appropriate long runs, and then measuring their respiration every seven days until a major change in respiratory behavior could be detected. Secondly, respiratory change following long-term starvation was demonstrated by monitoring respiration up to three times every 24 hours at field ambient water temperature at the time of collection for a four to five day period following collection. Thirdly, response to low oxygen levels was followed by comparing normal respiratory behavior both to initial respiratory rates and to rates with decreasing oxygen tension of post-anaerobic snails. Finally the mortality due to sustained low oxygen stress was determined for each population (FH, CAN, ECF) by placing 20 snails in glass stoppered bottles containing deoxygenated field water maintained at field temperatures. Periodically, the number of snails still alive was recorded, and the time at which 50% of the snails died from low oxygen stress (LD-50) is presented below.

All respiratory rate values were computed as a rate per milligram "shell-free" dry tissue weight per hour (μl O_2/mg/hr). In this case, "shell-free" dry tissue weight equals the dry weight of the appropriate size-class minus the dry weight of its shells. Shell dry weights had been determined from a regression of dry weight against aperture length for each population.

Results

Respiration experiments fall into three general classes: seasonal measurements of short-term respiration, temperature acclimation studies, and more specific investigations of low oxygen stress. Within each experimental set of size groups there was no consistent change in respiratory rate with change in mean dry tissue

weight per animal. A similar lack of correlation between size and respiration rate has been reported for *Physa hawnii* (Daniels and Armitage, 1969). Therefore, oxygen uptake rates of all size groups in each experiment were averaged for each experimental temperature.

The experiments in which respiration rates were recorded over long periods of starvation showed that the variation in oxygen consumption rates resulting from starvation falls within the normal range of variation found in any single experiment. The consistent long-term effect of starvation on respiration rate reported for the European stream limpet *Ancylus fluviatilus* by Berg *et al.* (1958) does not occur in *Laevapex*.

Respiration rate in *Laevapex* is closely correlated to field water temperatures. During the winter when water temperatures drop to between 0°–2° C at all three sites, the respiration at ambient temperature and 100 per cent oxygen tension of overwintering specimens of *Laevapex* is correspondingly low (Fig. 1). For the months of January through March the average respiratory rate for CAN limpets was 0.223 $\mu l O_2$/mg/hr in 1970 and 0.161 $\mu l O_2$/mg/hr in 1971 (Fig. 1). The average winter rates for FH-L snails (FH-L is the overwintering generation of the bivoltine Sterling Pond population; see McMahon, 1972 and McMahon *et al.*, 1974) were 0.191 $\mu l O_2$/mg/hr in 1970 and 0.183 $\mu l O_2$/mg/hr in 1971 (Fig. 1). The ECF population had an average winter rate of 0.291 $\mu l O_2$/mg/hr during the months of March and November in 1971 (Fig. 1).

As temperature rises with the onset of spring, the respiration rate of the limpets shows a corresponding increase. Peak rates of respiration at 100 per cent oxygen tension and ambient temperature occur in July and August when field water temperatures are the greatest (25° C–30° C). At ECF, average summer respiration rates during July and August in 1971 were 2.010 $\mu l O_2$/mg/hr (Fig. 1). The CAN limpets had similar average rates of 1.818 $\mu l O_2$/mg/hr in 1970 and 1.759 $\mu l O_2$/mg/hr in 1971 (Fig. 1). The FH-E *Laevapex* population (FH-E is the spring generation of the bivoltine Sterling Pond population) had a very high average summer respiratory rate in 1970 of 7.312 $\mu l O_2$/mg/hr and a lower rate of 1.939 $\mu l O_2$/mg/hr in 1971 (Fig. 1). The lower summer rate in 1971 at FH may have resulted from the unusually low over night temperatures occurring that summer. Since Sterling Pond water temperature is normally within a few centigrade degrees of air temperature the 1971-E *Laevapex* population my have received a series of cold shocks which kept respiration rates at ambient temperature lower than usual.

Taken from each population in mid-summer, three groups of twenty specimens were held in pond water depleted of all oxygen at ambient temperature. The mean 50% mortality times (corresponding to LD-50's) for each set of sixty animals are: ECF, 27° C, LD-50 = 6 hours and 35 minutes; CAN, 23° C, LD-50 = 16 hours and 50 minutes; and FH, 24° C, LD-50 = 16 hours and 50 minutes. The overall mean LD-50 time for summer limpets in all three populations is 14 hours and 5 minutes. Similar values have been reported for other freshwater snails (Von Brand, Baernstein, and Mehlman, 1950).

After treatment with low oxygen stress in water depleted of all oxygen (zero oxygen tension) all limpets showed increased resipratory rates which were maintained for long periods. ECF limpets collected April 20, 1971 and held in water completely depleted of oxygen (zero O_2 tension) at 20° C for 48 hours showed

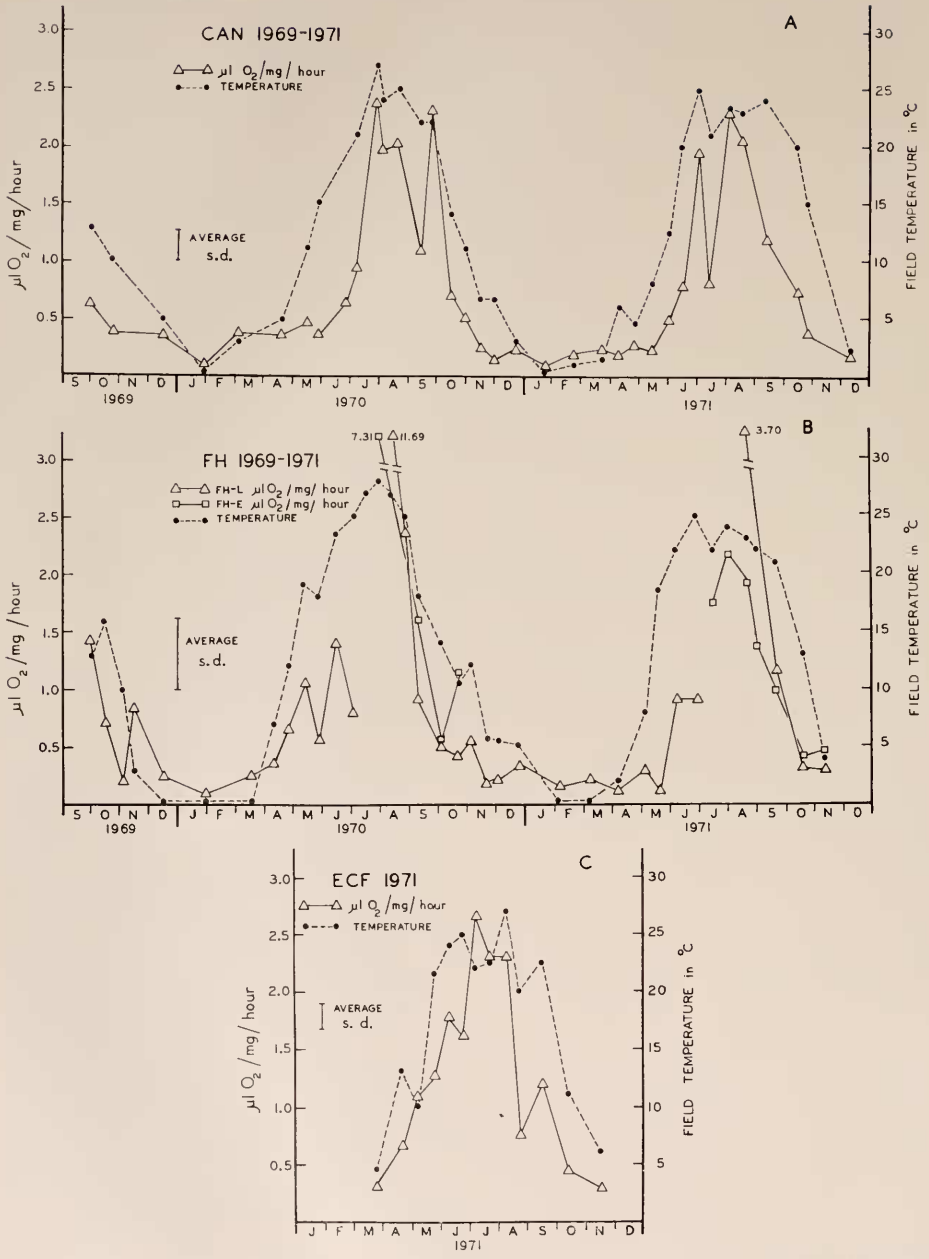

FIGURE 1. Respiration rates at ambient temperature. Oxygen consumption rates are in microliters oxygen per milligram shell-free dry tissue weight per hour at ambient field temperature. Open triangles, connected by solid lines, represent respiration rate and solid circles, connected by dotted lines, represent ambient field temperature (A, CAN; and B, FH, from late 1969 through 1971; C, ECF, in 1971). The points represent mean values of oxygen consumption rates for one to five separate groups of snails. The average s.d. indicates an average

TABLE I

Mean midwinter and midsummer respiration rates at 10° and 20° C and 100 per cent oxygen saturation

		Winter January–March			Summer June–August		
		Average respiration rate in $\mu l O_2$ mg hr	Range	Q_{10}	Average respiration rate in $\mu l O_2$/mg hr	Range	Q_{10}
FH-L	20°C	0.554	0.48–0.62	2.01	1.338	0.69–2.56	1.48
	10°C	0.276	0.22–0.33		0.906	0.33–2.03	
CAN	20°C	0.553	0.48–0.69	2.38	1.277	0.33–2.24	1.26
	10°C	0.232	0.20–0.30		1.017	0.31–2.01	
ECF	20°C	0.917	0.84–0.99	2.28	1.503	0.74–2.20	1.76
	10°C	0.402	0.24–0.57		0.856	0.30–1.42	

no subsequent decrease in respiratory rate (from these increased rates) for 189 minutes after return to oxygen saturated water. A similar group of ECF limpets collected May 24, 1971, and exposed to oxygen depleted water (zero O_2 tension) for 37 hours showed no detectable short-term change in these increased rates after return to fully saturated water. Both groups of limpets showed just over a 100 per cent increase in respiratory rate immediately after treatment at all O_2 tensions when compared to normal rates before low oxygen stress (Fig. 2). The ECF limpets collected April 20, 1971 had respiration rates with decreasing oxygen tension re-measured 16 hours and 135 hours after return from low oxygen stress treatment to fully saturated water (Fig. 2A). This figure shows that the limpets appear to maintain their increased rates of respiration for more than 135 hours after they had been returned to water at 100 per cent saturation.

The ECF limpet group collected May 24, 1971 was expected for a second 43 hour period to oxygen depleted water (zero oxygen tension), following the first 37 hour low oxygen treatment and respiration measurement. Figure 2B shows that this second treatment increased respiration at all oxygen tensions to more than 4 times the pretreatment rates, and to twice that of the rates recorded after the first treatment.

Figure three shows the average respiratory rate of specimens of *Lacuapex* at 100 per cent saturation at 10° C and 20° C throughout the years 1970 and 1971 for the three populations. During the winter months, when environmental water temperatures are between zero and five degrees centigrade, at 10° C and 20° C the limpets' respiration rates are very low. As water temperatures rise rapidly in June, the respiration rates also increase and they remain throughout the summer at more than twice the winter average (Fig. 3). Table I sets out the average winter and summer rates for all three *Lacuapex* populations for the year 1971.

of the standard deviations for all points which represent a mean of three or more separate determinations.

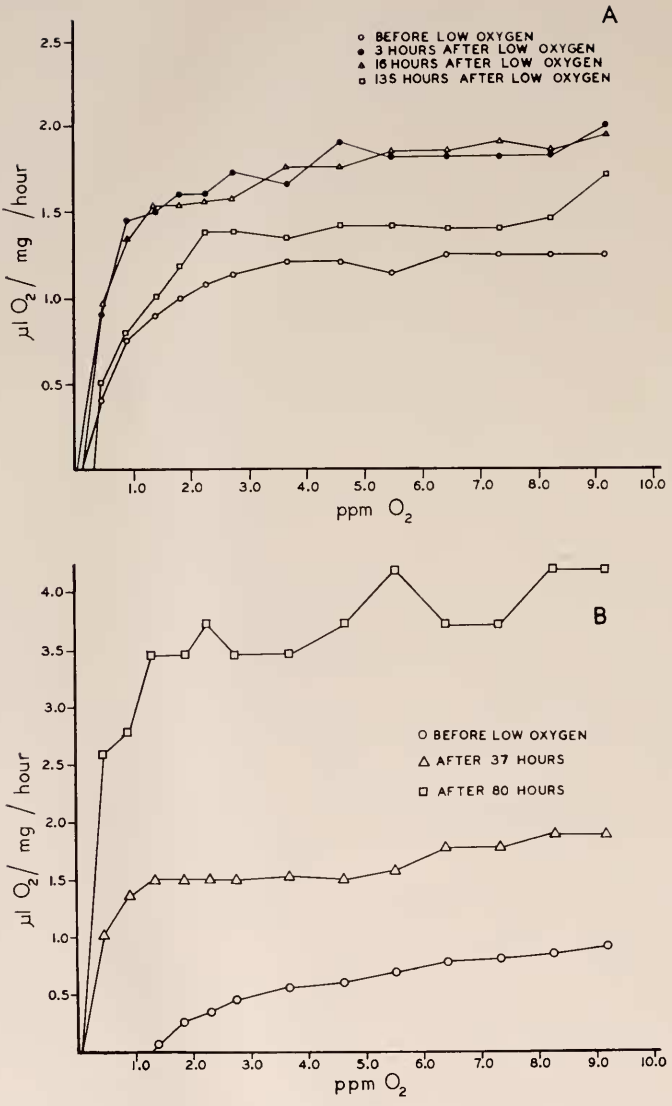

FIGURE 2. Respiration rate in microliters oxygen per milligram shell-free dry tissue weight per hour is plotted against oxygen tension in parts per million oxygen decreasing from right to left: (A) rate of oxygen consumption after low oxygen stress at 20° C. Limpets which have not been stressed with low oxygen conditions are shown as open circles. The solid circles represent respiration for the same limpets three hours after return to fully oxygen-saturated water from 48 hours of low oxygen stress; the triangles, 16 hours after return to fully saturated water from 48 hours of low oxygen stress; and the squares, 135 hours after return to fully saturated water from 48 hours of low oxygen stress; and (B) the effect of long-term low oxygen stress on oxygen consumption at 20° C. Limpets which have not been stressed with low oxygen conditions are indicated by circles. The triangles represent respiration rates for the same limpets after 37 hours in oxygen depleted water and the squares represent their respiration after 80 hours in oxygen depleted water. (Note that at 20° C and standard pressure, full saturation equals 9.17 ppm O_2.)

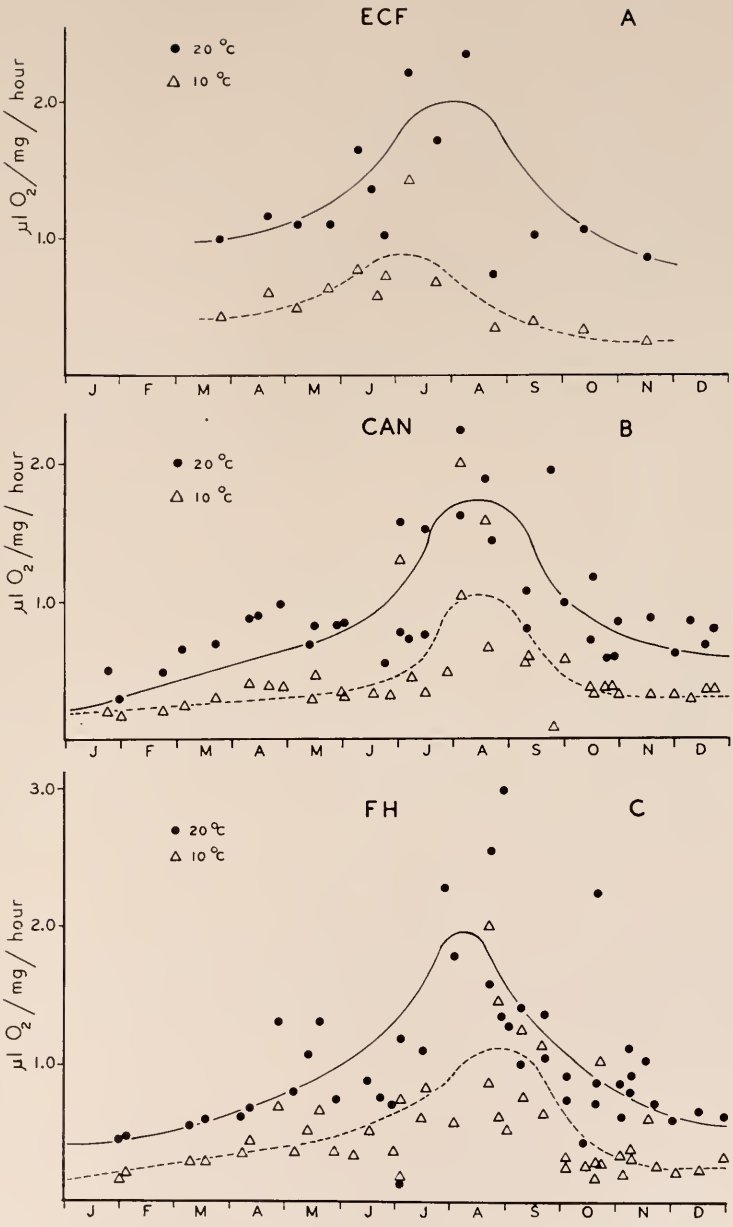

FIGURE 3. Rate of oxygen consumption at 10° C and 20° C throughout the year. Circles are oxygen consumption rate in microliters oxygen per milligram shell-free dry tissue weight per hour at 20° C and the triangles the rate at 10° C and at 100% oxygen tension. Acclimation is shown by the increase in rate during the summer months in the ECF (A), CAN (B) and FH (C) populations. Such a respiratory pattern has been previously called "reverse acclimation." For further explanation see text. The lines drawn on the three figures are purely arbitrary and assist only in the visualization of trends. The points represent mean values of oxygen consumption rates for one to five separate groups of snails.

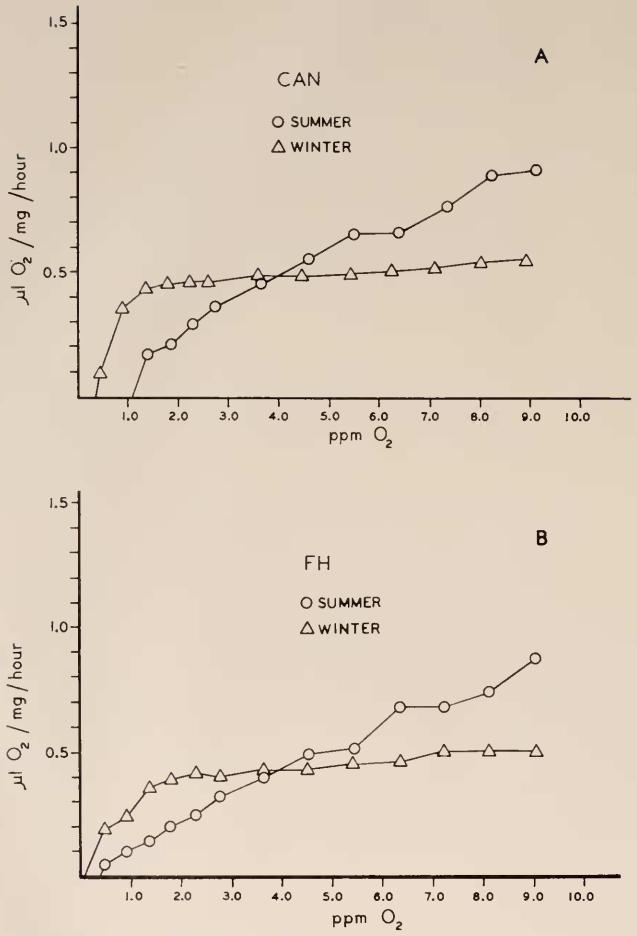

FIGURE 4. Respiratory acclimation. Oxygen consumption rate in microliters oxygen per milligram shell-free dry tissue weight per hour is plotted for decreasing oxygen tension at 20° C from right to left, for winter-conditioned (triangles) and summer-conditioned (circles) specimens of *Laevapex* from CAN (A) and FH (B). Note that at 20° C and standard pressure, full saturation equals 9.17 ppm O_2.

When respiratory rates are measured at any intermediate temperature, many poikilotherms which have been maintained at a lower temperature show a higher respiration rate than similar animals previously maintained at a higher temperature. This respiratory response to temperature conditioning over periods of about 7–20 days is called "Respiratory Acclimation." When the respiration rate of specimens of *Laevapex* from all three populations is followed at 10° C and 20° C at 100 per cent oxygen tension, limpets from a summer population accustomed to high ambient water temperatures have a greater respiratory rate than limpets from winter-conditioned populations accustomed to low ambient water temperatures when oxygen consumption is measured at the same temperatures (10° C and 20° C). This type of

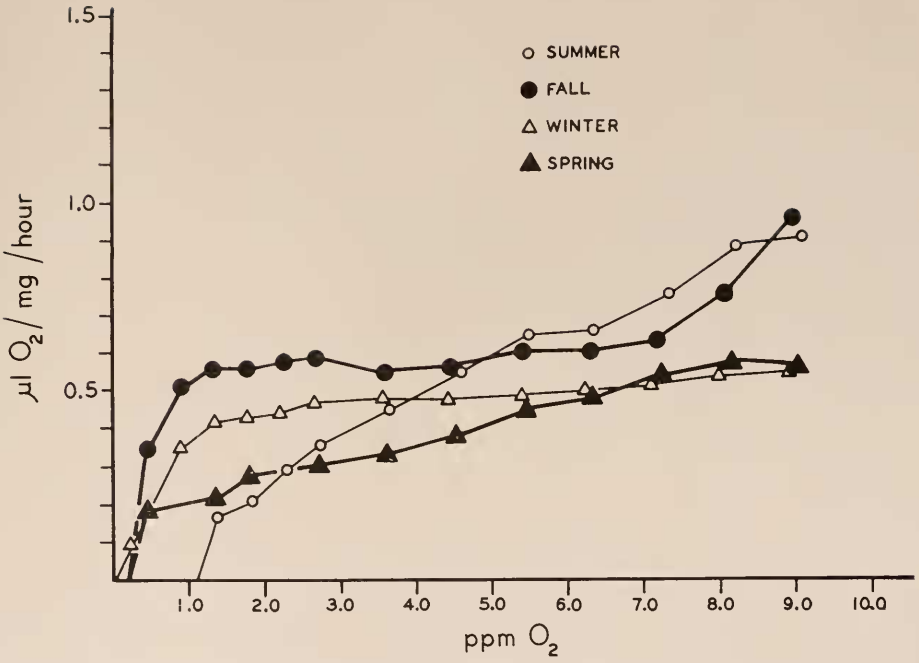

FIGURE 5. Respiratory acclimation with change in seasons. Respiration rate in microliters oxygen per milligram shell-free dry tissue weight per hour is shown with decreasing oxygen tension at 20° C from right to left, for limpets from the CAN population taken in midwinter (open triangles), spring (solid triangles), midsummer (open circles), and fall (solid circles). Note that at 20° C and standard pressure, full saturation equals 9.17 ppm O_2. The most obvious shifts from summer to fall and from winter to spring concern the left hand parts of the curves, that is, they are shifts in the limpets' responses to low oxygen tensions.

acclimation has previously been called "reverse acclimation." Similar respiratory acclimation has been reported for seven other invertebrates and one fish, and these instances will be listed and discussed below.

Long-run respiration experiments on *Laevapex* from these three populations show seasonal variation in respiratiory rate at low oxygen tensions. Summer-conditioned snails have higher initial respiratory rates at 100 per cent oxygen saturation at 20° C than (otherwise similar) winter-conditioned snails. However, summer-conditioned specimens of *Laevapex* are oxygen dependent in their respiration; as oxygen tension decreases in the respiration chamber, respiration rate also decreases (Fig. 4). Winter-conditioned limpets have lower initial oxygen uptake rates at 100 per cent oxygen saturation at 20° C, but these rates are maintained from 100 per cent to 10 per cent oxygen saturation and only after oxygen tension falls below 10 per cent is there any appreciable decrease in respiratory rates (oxygen independent respiration) (Fig. 4). Therefore, at lower oxygen tensions (below 40–50% O_2) specimens of *Laevapex* show a normal acclimatory response to temperature. The respiration rate of winter-conditioned animals is higher than that of summer-conditioned animals at these low tensions below 40–50% O_2 when mea-

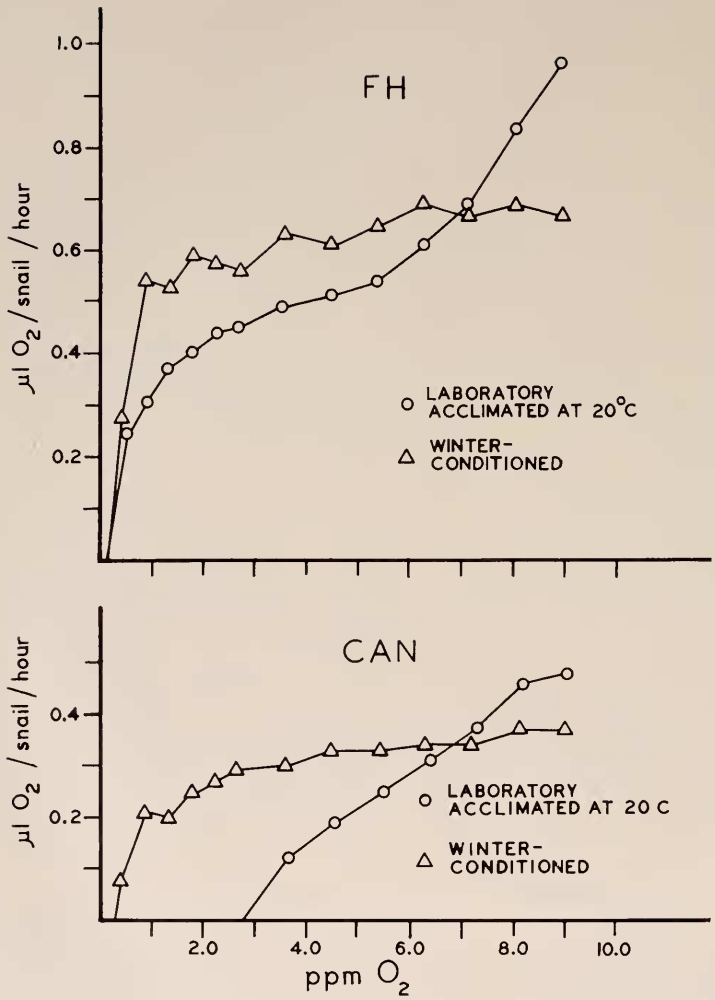

FIGURE 6. Respiratory acclimation in the laboratory. The vertical axes show respiration rate in microliters oxygen per snail per hour with decreasing oxygen tensions at 20° C from right to left, for winter-conditioned limpets from CAN and FH (triangles), and for the same limpets after being maintained in the laboratory at 20° C and 100% oxygen tension for 12 (CAN) and 20 (FH) days. Note that at 20° C and standard pressure, full saturation equals 9.17 ppm O_2.

sured at 20° C. Berg and Ockelmann (1959) investigated respiration rate with decreasing oxygen tension in nine freshwater snails. The respiration rates of the eight species were significantly diminished with decreasing oxygen tensions. Only *Bithynia leachi* showed an oxygen independent respiratory pattern similar to that of winter-conditioned *Lacvapex*.

The snails shift from the summer to winter respiration pattern in the fall gradually as water temperature begins to drop and they gradually return to the

summer respiration pattern in the late spring when water temperatures begin to rise. Figure 5 shows intermediate spring and fall respiration rate curves against oxygen tension at 20° C for the CAN population. These curves demonstrate the slow shifts which occur in these respiratory patterns.

Figure 6 shows respiration rate at 20° C plotted against oxygen tension for limpets collected from Canandaigua Outlet on March 21, 1971 (ambient water temperature 1.5° C) and from Fair Haven on April 6, 1971 (ambient water temperature 2.0° C). Both groups were kept at ambient temperature until the experiments were started. They both had typical winter curves with respiration rate decreasing only slightly as oxygen tensions decreased. After the initial long-run experiment the limpets were kept in an incubator at 20° C and at 100 per cent oxygen saturation (about 9.17 mgO_2/l). The respiration rate with decreasing oxygen tension was checked every three to four days thereafter until the limpets shifted to the summer respiratory pattern (Fig. 6). At 20° C and 100 per cent oxygen saturation, acclimation to the summer pattern took the CAN limpets 12 days and FH limpets 20 days to complete. The possible adaptational significance of these acclimation patterns will be discussed later.

Discussion

Annual variation of respiration rate at ambient temperatures have not been reported in detail for any mollusc populations. One Norwegian population of the oyster, *Ostrea edulis,* was shown to have a midsummer respiratory rate at 25° C 19 times greater than its mid-winter value at 0.0° C (Pederson, 1947). In two Danish populations, the European stream limpet, *Ancylus fluviatilis,* increases its repiratory rate during the summer temperature maxima by five to six times in one population (maximum at 13° C and minimum at 3° C) and in the other by 2.8 times (19° C and 7° C) their winter minimum rates (Berg, *et al.,* 1958). Burky (1969 and 1971) reported that maximum summer respiration of *Ferrissia rivularis* at 17° C was 10.5 times greater than the minimum winter value at 0.0° C. In the three populations of *Lacvapex* of the present study FH-L had a 1971 maximum summer rate 36.0 times (0° C and 25° C) greater than the minimum winter rate (in 1970 this value was abnormally high at 127.1) (0° C and 28° C) while the value was 9.4 (4.5° C and 27° C) for ECF in 1971 and 26.6 (0° C and 26° C) and 26.2 (0° C and 25° C) in the CAN-70 and CAN-71 populations (Fig. 1). These differences in winter and summer respiration are considerably greater than those for *Ancylus fluviatilis* or *Ferrissia rivularis.* Since the change in respiration rate in specimens of *Lacvapex* corresponds in part to change in ambient temperature, the large annual variation in ambient water temperature as recorded at the three sites may account for the large annual variation in respiration rates (Fig. 1). Except for the high respiration of the FH summer limpets in 1970 no significant differences were found between any of the three populations in respiration rate at corresponding ambient temperature.

Prosser (1955, 1967) and Bullock (1955) have reviewed acclimation in poikilothermic animals, and Segal (1961) has described some cases of acclimation in molluscs in great detail. Although the majority of poikilotherms including molluscs which have been investigated show normal acclimation (Bullock, 1955; Segal, 1961) reverse acclimation at 100% oxygen tension has been reported for

natural overwintering populations in the goldfish (Roberts, 1960, 1966), in two species of barnacle (Barnes, Barnes and Finlayson, 1963) and in a freshwater gammarid (Krog, 1954). Among molluscan populations, reverse acclimation has been described for: a high intertidal limpet, *Patella vulgata* (Davies, 1965); a terrestrial pulmonate, *Helix pomatia* (Blazka, 1954); two European ancylid pulmonates, *Ancylus fluviatilis* and *Acroloxus lacustris* (Berg, 1951, 1952, 1953; and Berg *et al.,* 1958); and in the North American stream limpet *Ferrissia rivularis* (Burky, 1969, 1970, 1971). Two different interpretations of the adaptive significance of reverse acclimation to a particular species have appeared in the literature. The first hypothesis is that the reduction of respiration rate during the winter is an adaptation to the depletion of oxygen resulting from ice cover and subsequently reduced surface diffusion of oxygen (Roberts, 1960, 1966; Krog, 1954). These animals are said to be lowering their metabolic demand for oxygen as a response to its reduced availability in the environment. The other interpretation of the function of reverse acclimation is that in the winter when primary productivity is at a minimum and the animal is not actively feeding, then lower winter metabolic rates (reflected in decreased respiration rates) act to conserve energy stores during the overwintering period (Burky, 1969, 1971). Both of these interpretations involve paradoxes for the overwintering populations of *Laevapex* studied. *Laevapex,* like *Ferrissia* (Burky, 1971) shows an increase in total carbon and in C:N ratio just prior to overwintering (McMahon, 1972). As the majority of gastropods have been demonstrated to store carbohydrates (von Brand *et al.,* 1950; Goddard and Martin, 1966; Russell-Hunter, Meadows, Apley and Burky, 1968; and references within), this carbon increase in fall populations is assumed to be the result of a buildup of carbohydrate storage products before overwintering. But, just prior to winter, specimens of *Laevapex* crawl down rocks, into reducing mud where oxygen tensions are extremely low.

In poikilothermic animals which remain inactive during the winter, reverse acclimation to temperature is a paradoxical phenomenon (Prosser, 1955). To remain active at low temperatures, poikilotherms normally have to increase their metabolic rate over that of warm-acclimated animals of the same species (Prosser, 1955, 1967; Bullock, 1955).

Conservation of energy stores by reverse acclimation also presents a paradox. All previous reports of reverse acclimation, and my own results for initial respiratory rates, are based on respiration rates determined at 100% oxygen tension at the different experimental temperatures. In nature, *Laevapex* and other organisms can live in waters of very low oxygen tension. As is well known, anaerobic metabolism is highly inefficient and requires a much greater energy flow than does aerobic metabolism to provide an equal amount of energy output (von Brand *et al.,* 1950; Goddard and Martin, 1966, and references within). Under conditions of low oxygen tension in these inactive winter populations reverse acclimation of already low winter respiratory rates could make the animals partially anaerobic and highly inefficient in the metabolism of their energy stores. As is the case with *Laevapex,* in at least three previously reported cases of reverse acclimation: *Gammarus* (Krog, 1954); *Acroloxus laustris* (Berg, 1952); and goldfish (Roberts, 1960, 1966) the animals overwinter in oxygen depleted waters. Therefore, reverse respiratory acclimation as has been described at full oxygen saturation

appears certainly no adaptation to temperature alone. Rather, reverse acclimation is merely a manifestation of a more general acclimatory change in the respiratory behavior of these animals from oxygen-dependent respiration at high temperatures to oxygen-independent respiration at low temperatures. This shift allows winter-conditioned animals to have a higher rate of respiration than summer-conditioned animals would have in such low oxygen winter environments. Such a respiratory shift is triggered by the decrease in water temperature in the fall and the shift conditions the animals to oxygen-independent respiration for their winter environment. Thus, temperature change is merely the signal for the acclimatory shift and is not the adaptationally significant factor. This new hypothesis is largely confirmed by the results of the respiration experiments with decreasing oxygen tension which show that in the summer when the limpets are active and in areas of high O_2 concentration they have higher initial respiratory rates at 100% oxygen saturation at 20° C than do winter-conditioned limpets. However, the respiration rate of summer-conditioned specimens of *Lacuapex* is oxygen-dependent and decreases proportionately with decreasing oxygen tension (Fig. 4). Winter-conditioned limpets have lower initial oxygen uptake rates at full oxygen saturation at 20° C, but their respiration rate is independent of oxygen concentration and nearly the same oxygen uptake rate is maintained from 100% to 10% oxygen tension at 20° C. Only after oxygen tension falls below 10% (less than 1.0 mgO_2/l at 20° C) is there any appreciable rate decrease (Fig. 4). Therefore, given the lower oxygen tensions of reducing mud, the acclimatory processes of overwintering specimens of *Lacuapex* could be described as positive rather than negative or reverse. The respiration rate of winter-conditioned animals is higher than that of summer-conditioned animals when both are measured at these low oxygen tensions (below 4.0 mgO_2/l) and at 20° C. This increase in respiratory rate at low oxygen tensions would seem to be an adaptation which allows *Lacuapex* to metabolize aerobically and make efficient use of its carbohydrate energy stores during the period of winter inactivity.

Anaerobic metabolism in freshwater snails has been reviewed by von Brand (1946), and more recently by Goddard and Martin (1966). Most of the freshwater snails studied have been reported to repay an oxygen debt after experiencing periods of anaerobiosis (von Brand and Mehlman, 1953; Goddard and Martin, 1966; and references within). In contrast, the present work on *Lacuapex* shows no overt repayment of an oxygen debt as it is normally described, even after long exposure to waters of very low oxygen concentration. Instead, exposure to oxygen depleted water in *Lacuapex* seems to cause a general increase in respiratory rate at all O_2 tensions. This increased rate is maintained for at least 135 hours after return of the limpets to fully saturated conditions, and appears in part to be short-term acclimation of respiratory rate to low O_2 tensions. Prosser (1955) points out that some acclimatory shifts are accomplished in only 17–24 hours. In 18 species of freshwater snails von Brand, *et al.*, (1950) found that anaerobic metabolism resulted in temporally increased levels of lactic acid. But, while lactic acid was retained in the tissues of all physids and lymnaeids tested, it was the major end-product of anaerobiosis in only two species. In contrast, in the planorbids and operculates tested, lactic acid was continuously excreted and there was no build-up (von Brand *et al.*, 1950). Specimens of *Lacuapex* increase their total

carbon content and C:N ratio prior to overwintering (McMahon, 1972). Such stores are probably carbohydrates (Russell-Hunter *et al.*, 1968; Goddard and Martin, 1966; and references within) and may be utilized to survive periods of extremely low oxygen tension encountered by overwintering snails. Such snails migrate into reducing mud. In this respect *Laevapex* is much like the goldfish which also shows reverse acclimation (Roberts, 1960, 1966). Specimens of the crucian carp taken from winter habitats of low oxygen tension were shown to pay no normal oxygen debt after long periods of anaerobiosis. Instead of lactic acid, fat and higher fatty acids appeared to be the end-products of an anaerobic metabolism (Blazka, 1958). Similarly, of 11 freshwater snails, those with low resistance to anaerobiosis were shown to accumulate lactic acid in the blood during low oxygen stress, while those more resistant to low oxygen stress appear to accumulate less toxic higher fatty acids (Mehlman and von Brand, 1951). Freshwater snails under anaerobic stress will excrete lactic acids and the other end-products of metabolism (von Brand *et al.*, 1950; von Brand and Mehlman, 1953). Because of the small tissue volume of *Laevapex* most possible end-products of anaerobic metabolism could be readily diffused to the environment or actively excreted. Without metabolite accumulation during anaterobiosis, there need be no overt payment of an oxygen debt on return to oxygen saturated water.

I wish to express my gratitude to Dr. W. D. Russell-Hunter for his assistance and guidance in all phases of this work.

Summary

1. From three populations of *Laevapex fuscus* samples were collected bi-weekly through 1970 to 1972. Oxygen consumption rates at ambient temperature and at 10° C and at 20° C were determined. Other respiration experiments involved decreasing O_2 tension at 20° C and the consequences of low oxygen stress.

2. At ambient temperatures, respiration during the summer is normally 26 to 36 times the winter respiratory rate.

3. After treatment with low oxygen stress there is no overt payment of an oxygen debt in *Laevapex*, instead there is a general increase in respiratory rate at all oxygen tensions. These rates are maintained for up to 135 hours after return to fully saturated conditions and appear to be in part a short-term acclimation of respiratory rates to low oxygen tensions.

4. Measured at 100% oxygen tension at 10° and 20° C, respiration rates are higher in the summer than in the winter for all three populations. Such a respiratory response to temperature has been called reverse acclimation. *Laevapex* lives in conditions of low oxygen tension during the winter. The respiration experiments with decreasing oxygen tension show that at low oxygen tensions winter-conditioned specimens have a *higher* respiratory rate than summer-conditioned individuals. Such a response to low oxygen tensions may be interpreted as a positive rather than reverse acclimation.

5. A hypothesis is advanced regarding "reverse" acclimation in this species. Reverse acclimation appears to be merely one manifestation of a more general accli-

matory change in winter-conditioned *Laevapex*. These limpets, when winter-conditioned, have a higher respiratory rate at low oxygen tension than do summer-conditioned limpets. Although this acclimatory process appears to be triggered by decreasing temperature, its adaptational significance involves the survival of the inactive overwintering limpet in contact with reducing mud.

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